

Comments on "Estrogenicity of Resin-based Composites and Sealants Used in Dentistry"

Considerable concerns about bisphenol A (BPA) as a potential endocrine disruptor remain controversial among dentists and patients due to the study of Olea et al. (1). Their study has frequently been mentioned in scientific papers as well as in the mass media, popular journals, and books. When I read the paper, I was shocked to find unscientific data related to high performance liquid chromatography (HPLC) analysis. I am concerned about continued reference to this study. Ashby (2) commented on the study in a letter in *EHP* titled "Bisphenol-A Dental Sealants: The Inappropriateness of Continued Reference to a Single Female Patient," but I feel his comments are insufficient.

Olea et al. (1) reported that 90–931 µg of BPA was identified in saliva collected from 18 subjects treated with 50 mg of a bisphenol A diglycidyl methacrylate (bis-GMA)-based sealant on molars during a 1-hr period after treatment. However, based on data presented in their Table 1, this is unlikely. According to Table 1 (1), 50 mg of the sealant should contain 3.7 µg of BPA or 78.7 µg of a mixture of bis-GMA, bisphenol A diglycidyl ether (BADGE), bisphenol A dimethacrylate (BPDMA), and BPA. This mixture (78.7 µg) is equivalent to 48.2 µg of BPA. Assuming that all the components of the mixture leached into the saliva uncured and were degraded completely to BPA within 1 hr, the amount of BPA collected from the saliva should be 48.2 µg. However, their Table 2 (1) showed 1.9–19.3-fold higher values than expected (89.9–931.0 µg).

In the "Results," Olea et al. (1) reported that

A subject initially selected for treatment had been treated with tooth sealant 2 years earlier; chromatograms demonstrated the presence of bisphenol-A (66.4 µg) and bisphenol-A dimethacrylate (49.2 µg) in her saliva before the second treatment. The results from this subject were excluded from analysis.

This finding suggested that 66 µg/hr of BPA may be continually released over 2 years. This is unlikely because the treatment would have been practically impossible. Assuming that 66 µg of BPA leached into the saliva daily, leaching of BPA would have constituted 48.2 mg over 2 years. The BPA content in the sealant was 0.0074% according to their data. Therefore, the original amount of sealant used would have been about 650 g. Even if BPDMA, which is easily hydrolyzed to

BPA, is included in the calculation, it would have been approximately 200 g.

In their Table 1, Olea et al. (1) indicated that the amount of ethanol-soluble components in the composite resins and the sealant was 0.03–0.19% (calculated from the total weight of four components shown in Table 1 contained in 100 mg of commercial product). These values are much too small because commercial resins usually contain 15–50% of ethanol-soluble components. Olea et al. (1) stated that

these commercial formulations contain a large proportion of inorganic filler particles (50–85% by weight of the composite).

Moreover, they did not refer to triethylene glycol dimethacrylate (TEGDMA), which is a major component in all these commercial products. Assuming that their data were correct, some questions arise: *a*) BPA contents in the ethanol-soluble components [most of which consist of a mixture of bis-GMA (and BPDMA) and TEGDMA] were much too high—1.7–35.2% (calculated from the ratio of BPA to the total weight of four components). Such impure monomers are unlikely in the chemical industry at the present time. BPA may exist in the monomers as an impurity. *b*) The total amounts of ethanol-soluble components markedly increased or decreased in many cases after hydrolysis in alkaline or acidic media beyond the values expected from the contents before hydrolysis. This should not occur in a chemical reaction; the reaction should follow the law of conservation of mass. *c*) The increase in the BPDMA content in alkaline media for composites 1 and 2 is unlikely because it is easily hydrolyzed to BPA in the media. *d*) The marked increase in bis-GMA content after hydrolysis in acidic or alkaline media, composite 3, is unlikely because bis-GMA is hydrolyzed under such conditions.

The calculation presented in Olea and colleagues' Table 2 (1) is difficult to understand. The saliva volume multiplied by the concentration of BPA should be equal to the total amount of BPA in saliva. None of the data presented in their Table 2 supported this arithmetic.

Olea et al. (1) may have misunderstood that BPA (as well as BADGE) constitutes components of resins, judging from the statement in the first paragraph of the "Discussion":

... we demonstrate that [BPA] and [BPDMA], components of commercial resin-based composites and sealants used in dentistry, are estrogenic

BPA and BADGE are not components, but only a trace amount of these materials

is contained as impurities in bis-GMA and/or BPDMA monomers. Therefore, the amount of leached BPA is extremely small or not detectable, as reported recently by other investigators (3–5). On the other hand, BPDMA is a component of the sealant used in their study. Therefore, leaching of a small amount of unpolymerized BPDMA is likely (3,4). Furthermore, the detection of BPA derived from BPDMA in saliva is likely because BPDMA is easily hydrolyzed to BPA in saliva (6). Thus, it is reasonable to assume that most of the BPA identified in saliva by Olea et al. (1) can be attributed to leached BPDMA. Therefore, the use of BPDMA should be extensively examined.

Data relevant to HPLC analysis presented by Olea et al. (1) are not reliable. These data should be corrected or withdrawn, and further reference to this data should be avoided.

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Olea's Response

Imai seems to be upset about our recommendation to curtail the use of bisphenol A-based sealants and claims to have found unscientific data among our HPLC results. His opinions appear to be based on a poor reading of our study (1) and what he refers to as "relevant papers." Unfortunately, Imai reveals no data of his own and reports no experiments or time spent actually trying to solve this problem.

Three of the references provided by Imai are only abstracts (2–4), one in Japanese, precluding a full reading and critical analysis. The article by Nathanson et al. (5) elicited a detailed response from our group in the form of a letter, which the editor of the *Journal of the American Dental Association (JADA)* declined to publish, despite our insistence and repeated submission of ever shorter versions.

Thus, we consider this an appropriate opportunity to respond to both Imai and Nathanson, given the importance of our findings for the practice of dentistry and as a public health issue. The work by Nathanson et al. (5) was largely devoted to refuting our paper on the estrogenicity of pit and fissure sealants (1). Their claim that their findings on leachability contradict our results implies that the two studies are comparable, while in fact their study differs substantially from ours. First, Nathanson's paper (5) does not test the estrogenicity of sealant eluates, although the demonstration of estrogenic compounds leaching from a sealant was the most important finding of our study, a point that was also ignored by Imai. The nature of these compounds could be diverse because several components of commercial sealants have been identified as estrogenic xenobiotics. We postulated that bisphenol A and the dimethacrylate of bisphenol A (bisDMA) are candidates for this hormonal effect, but the presence of other chemicals contributing to this effect cannot be ruled out.

We reported that BPA leached from unpolymerized sealant after heating (100°C) and was found in the saliva of patients after *in situ* polymerization. Data we presented (Table 1) on the content of BPA, BADGE, and bisDMA in one sealant and three composites, of concern to Imai, are indicative of the presence of these compounds in the commercial samples. As Imai comments, an exhaustive analysis of these samples reveals that the values we reported "are much too small because commercial resins usually contain 15–50% of ethanol-soluble components." We did not study the entire content of components in the commercial samples, but we did identify the source of the monomers found in the saliva of treated patients. Most of the discrepancies asserted by Imai are due to his idiosyncratic reading of the experimental data. For example, we did not mention "ethanol-soluble components" for the simple reason that all the extracts tested were water soluble. Moreover, to estimate leaching over time by extrapolating from our results, as Imai attempts to do, is not warranted.

We chose to explore sealant polymerization within the mouth because this is where

polymerization is done in practice. In contrast, Nathanson et al. (5) polymerized the material *in vitro*. Success of the polymerization process is clearly dependent on the microenvironmental conditions: saliva, humidity, access to curing light, etc. These differences in experimental conditions prevent comparisons between the two papers. In fact, despite their different approach, Nathanson et al. (5) did show that bisDMA leached into the eluates of Delton (Dentsply Trubyte, York, PA) and Defender (Henry Schein, Inc., Melville, NY) sealants, and the amount of bisDMA leached by Delton sealant over 4 min (1.3 µg/mg of sealant) greatly exceeded the maximum amount recovered during 1 hr from the saliva of the three patients presenting detectable amounts of bisDMA (0.063 µg/mg of sealant) in our study. Nathanson et al. (5) found no BPA leaching from any of the sealants they tested. In contrast, we found BPA in the saliva of patients treated with Delton sealant at concentrations ranging from 1.8 to 18 µg/mg of sealant applied. In addition to the site of the polymerization process, other technical differences may account for these discrepancies. For example, Nathanson et al. (5) used a wavelength of 215 nm for the detection of sealant moieties separated by HPLC. Lineal dimethacrylate derivatives such as TEGDMA that absorb at this wavelength may be detected in this protocol. In contrast, by using a 280-nm wavelength, we detected BPA and other aromatic monomers such as BADGE, bisDMA, and bis-GMA, without interference from lineal ethyleneglycol dimethacrylate derivatives. Furthermore, Nathanson et al. (5) offer curious details. For example, in the "Material and Methods," they stated that the GC/MS technique was done using a "DB-5 HPLC column of 30 m." As far as we know, and as confirmed by J&W Scientific (Folsom, CA), no such column exists. It is difficult to assess what "Unknown I" peak represents because it is incorrect to "calculate the amount of Unknown I by using the BisGMA standard solution as the reference because their spectra are similar," as they did. The chromatographic data are inadequate because

- No calibration curves were shown for any of the compounds analyzed; moreover, the authors did not mention any parameters of these curves, neither fit nor correlation coefficients
- No indication of linearity of the detector response is presented
- No information about the accuracy of the chromatographic curve is provided
- The percentage of recovery for the standard curves is not reported
- The practice of confirming peaks by addition is of dubious reliability; instead,

GC/MS should be used to identify all unknown peaks.

Retention times in HPLC are not valid for identification. These technical shortcomings make it difficult to compare data.

We think that more data should be gathered before adopting the complacent position proposed by Nathanson et al. (5) and Imai. Recent observations have raised concerns about the estrogenicity of bisphenols (6–11): *a*) a more potent *in vivo* effect of BPA than that assessed by previous *in vitro* assays has been demonstrated (6,7); *b*) BPA seems to act on target organs other than breast and uterus (6,8–10); *c*) genetic differences in susceptibility to the estrogenic effect of BPA have increased concerns about human subpopulations with a higher sensitivity to this estrogen (6,11); and *d*) bisphenol A is just one of many compounds used by the plastics industry with demonstrated *in vitro* estrogenicity (12). By taking these studies into account, the American Dental Association may consider adopting a more realistic position regarding the use of bis-GMA-based composites and sealants, as recently suggested by Soderholm and Mariotti (13).

In short, the flaws in the paper by Nathanson et al. (5) undermine its conclusions on the safety of sealants; we regret the *JADA* Editor-in-Chief's decision not to publish our rebuttal to the allegations of Nathanson's group. Imai should not have used these flawed data to support his attack on our work. Dental health professionals ought to be aware of the potential risks of hormonally active compounds present in these formulations. Suppliers and other stakeholders should also be encouraged to address this public health issue. Until data are provided that challenge our data and arguments, the hypothesis of human exposure to estrogenic compounds leaching from bis-GMA-based composites cannot be withdrawn.

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Error in DEHP Background Concentration

In the May 1998 issue of *Environmental Health Perspectives*, Woodruff et al. (1) reported an analysis conducted as part of the U.S. Environmental Protection Agency (EPA) Cumulative Exposure Project (CEP). The EPA modeled air concentrations of chemicals listed in the Clean Air Act as hazardous air pollutants (HAPs) in over 60,000 census tracts and compared those concentrations to health benchmarks. The Phthalate Esters Panel of the Chemical Manufacturers Association has become aware of an error in the background concentration value used for bis(2-ethylhexyl) phthalate (DEHP), with the result that modeled air concentrations for DEHP—and thus the potential health hazard—were greatly exaggerated.

Woodruff et al. (1) reported, "Eight pollutants ... [including DEHP] had modeled concentrations exceeding the benchmark concentrations for cancer in 100% of the census tracts. For each of these HAPs,

the background concentration alone ... exceeded the benchmark concentration for cancer, as shown in Table 2." Their Table 2 (1) shows that the background concentration used for DEHP was 1.6 $\mu\text{g}/\text{m}^3$. That value was taken from Howard (2) who reported "mean remote ocean air concentrations" for DEHP of 0.07–0.17 ppb, citing Atlas and Giam (3). However, Atlas and Giam (3) actually reported remote DEHP air concentrations to be 0.32–2.68 ng/m^3 , with a mean of 1.4 ng/m^3 —a value more than 1,000 times less than the background value used for the CEP analysis. The panel has alerted the EPA to this error, and the EPA accordingly has corrected the CEP modeling report (4).

Table 2 of Woodruff et al. (1) shows that if the erroneous background value of 1.6 $\mu\text{g}/\text{m}^3$ is disregarded, the CEP model predicts DEHP air concentrations to exceed the health benchmark of 0.25 $\mu\text{g}/\text{m}^3$ in only 18 census tracts. Even this estimate probably exaggerates the potential health hazard for two reasons. First, to the panel's knowledge, the highest measured ambient DEHP air concentration in the United States that has been reported in the literature is 28 ng/m^3 (5)—an order of magnitude below the EPA's cancer health benchmark. Second, the EPA's health benchmark of 0.25 $\mu\text{g}/\text{m}^3$ was derived using an upper-bound unit risk methodology to extrapolate tumor data in rats and mice to human risk (6,7). However, numerous investigators now conclude that peroxisome proliferators such as DEHP pose little if any human cancer risk and that the quantitative risk assessment for such compounds should be based on a margin of exposure approach (8–10). This would significantly increase the health benchmark for DEHP and decrease (probably to zero) the number of census tracts in which modeled air concentrations would exceed the health benchmark.

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DEHP Correction

We thank Courtney Price for pointing out an error in the background concentration for bis(2-ethylhexyl)phthalate (DEHP) in our paper "Public Health Implications of 1990 Air Toxics Concentrations across the United States" (1). In this paper, emissions data from stationary and mobile sources are used in an atmospheric dispersion model to estimate outdoor concentrations of 148 toxic air contaminants for each of the 60,803 census tracts in the contiguous United States. Outdoor concentrations of air toxics were compared to previously defined benchmark concentrations for cancer and noncancer health effects. Benchmark concentrations are based on standard toxicological references and represent air toxic levels above which health risks may occur.

The results reported for DEHP are incorrect due to an error in the estimated background concentration for DEHP. We had originally used a value of 1.6 $\mu\text{g}/\text{m}^3$ for DEHP, which was reported by Howard (2). As pointed out by Price, Howard (2) had incorrectly reported the value from another source, Atlas and Giam (3). Consequently, we have revised the background concentration for DEHP to 0.0014 $\mu\text{g}/\text{m}^3$, consistent with the mean value reported by Atlas and Giam.

We had reported that the background concentration for DEHP was greater than the cancer benchmark for DEHP. However, the revised background concentration is much lower than the cancer benchmark. Thus, DEHP should not be included in the list of pollutants in Table 2 for which background concentrations alone exceeded cancer benchmark concentrations (1). The